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Opioid modulation of prolactin secretion induced by stress during late pregnancy. Role of ovarian steroids

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ABSTRACT

Background: The opioid system modulates prolactin release during late pregnancy. Its role and the participation of ovarian hormones in this modulation are explored in ether stress-induced prolactin release.

Methods/Results: Estrous, 3-day and 19-day pregnant rats were used. We administered the antagonist mifepristone (Mp) and tamoxifen to evaluate progesterone and estradiol action in naloxone (NAL, opioid antagonist) or saline treated rats. Ether stress had no effect on serum prolactin levels in controls but increased prolactin release in NAL-treated rats. Prolactin response to stress in NAL-treated rats was blocked by L-DOPA administration. Mp treatment on day 18 of pregnancy increased prolactin levels after stress without alterations by NAL. Tamoxifen on days 14 and 15 of pregnancy completely blocked Mp and NAL effects on prolactin release at late pregnancy. In contrast, stress significantly increased prolactin levels in estrous rats and pretreatment with NAL prevented this. On day 3 of pregnancy, at 6.00 p.m., stress and NAL treatment inhibited prolactin levels in saline-treated rat. No effect of stress or NAL administration was detected on day 3 of pregnancy at 9.00 a.m. *icv* administration of specific opioids antagonist, B-Funaltrexamine but not Nor-Binaltorphimine or Naltrindole, caused a significant increase in stress-induced prolactin release.

Conclusions: Opioid system suppression of prolactin stress response during late pregnancy was observed only after progesterone withdrawal, involving a different opioid mechanism from its well-established stimulatory role. This mechanism acts through a mu opioid receptor and requires estrogen participation. The opioid system and progesterone may modulate stress-induced prolactin release, probably involving a putative prolactin-releasing factor.

Introduction

The opioid system modulates prolactin secretion in non-pregnant, pregnant and lactating animals [1–6]. Dual neuromodulatory regulation of prolactin secretion by the opioid system during pregnancy was previously described [7]. After stimulatory action during the first days, a change to inhibitory control was established at the end of pregnancy, starting around day 16 [8]. Thus, both stimulatory and inhibitory actions of opioids, acting through different regulatory pathways, may result in an elevation of prolactin levels [7,9,10].

Administration of the antiprogesterone mifepristone (Mp) facilitates prolactin release by blocking the central inhibitory action of progesterone [7], and the effect of Mp can be enhanced by injecting the opioid antagonist naloxone (NAL). In fact, prolactin secretion during late pregnancy undergoes a paradoxical regulation by the opioid system in which progesterone plays an important role [7,8]. Moreover, Mp inhibits the hypothalamic dopaminergic neuronal system [11], the main inhibitory factor of prolactin secretion in terms of dopaminergic transmission and tyrosine hydroxylase (TH) expression [12,13]. This effect enables a

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significant activation of lactotrophs and primes the pituitary for a subsequent stimulatory action of NAL [14].

Several stressors may affect prolactin secretion [15–17], and endogenous opioid peptides participate in the prolactin response to stress [18–20]. Among the subtypes of opioid receptors (mu, delta and kappa receptors), the activation of mu opioid subtypes plays an important role in stress conditions [21,22]. Furthermore, ether stress induces a rapid increase in plasma prolactin concentrations in female, male, and androgenized rats [23], and ovarian steroids participate in this effect [24,25]. It is known that estradiol has a stimulatory effect on basal as well as on stress-induced prolactin release [26,27] and evidence suggests that progesterone may inhibit prolactin gene expression [28] and prolactin secretion [24] in response to stress. The mechanisms by which endogenous opioids and ovarian steroids may affect stress responsiveness are, however, still unclear.

Interestingly, hyporesponsiveness of the hypothalamus–pituitary–adrenal (HPA) axis to several stressors was described in late pregnancy [29]. Both endogenous opioids and progesterone, more specifically its metabolite allopregnanolone [30], seem to play an important role in the mechanisms involved in this suppressed HPA axis response [24,31].

Several changes occur in the maternal brain to prepare the different neuroendocrine systems involved in mechanisms regulating parturition and lactation [32,33]. Among others, endogenous opioids and progesterone play a role in maternal oxytocin and prolactin system adaptation [31,32].

The primary goal of this study was to examine the participation of the opioid system in the regulation of prolactin secretion in response to ether stress during late pregnancy and to establish a correlation with changes in ovarian steroids. Additionally, the ether stress response in other reproductive situations was evaluated, such as estrus day or day 3 of pregnancy where the stimulatory effect of the opioid system has been clearly established.

Materials and methods

Animals

Virgin female rats, 3 months old (200–220 g), bred in our laboratory and originally from the Wistar strain, were used. They were kept in a light (6.00 a.m. – 8.00 p.m.) and temperature (22 ± 2 °C)-controlled room. Rat chow (Cargill, Argentina) and tap water were available *ad libitum*. Vaginal smears were analyzed daily; virgin rats of 3 months of age showing two or three consecutive 4-day cycles were used on estrus day. Other groups of rats were made pregnant by being caged individually with a fertile male on the night of proestrus. Vaginal smears were checked for the presence of spermatozoa on the following morning; if positive, that day was considered day 0 of pregnancy (normal delivery on day 22 of pregnancy). Animal maintenance and handling were conducted according to the NIH guide for the Care and Use of Laboratory Animals (NIH publication N° 86-23, revised 1985 and 1991) and the UK requirements for ethics of animal experimentation (Animals Scientific Procedures, Act 1986). All experimental procedures were approved by the Care and Use of Laboratory Animals Committee (CICUAL) of the Faculty of Medical Sciences, National University of Cuyo, Mendoza, Argentina.

Surgical procedures

In pregnant rats receiving intracerebroventricular (*icv*) injections, stainless-steel guide cannulae were surgically implanted on day 12, 7 days before the experiment. The animals were anesthetized with a combination of xylazine hydrochloride (4 mg/kg) and ketamine hydrochloride (80 mg/kg) injected *ip* between 9.00 a.m. and 12.00 a.m. Rats were positioned in a stereotaxic frame and the stainless-steel guide cannula was inserted into the right lateral ventricle (M/L 1.5 mm, A/P-0.4 mm relative to bregma, 4.5 mm relative to dura [34]). Cannulae were fixed to the skull using dental acrylic and sealed until the time of drug injection. On the day of the experiment, 5 μl of specific antagonists were injected using a 10 μl Hamilton microsyringe connected to an injection needle that protruded 1 mm beyond the tip of the guide cannula placed in the lateral ventricle. Placement of cannulae was verified histologically at the end of the experiment.

Drugs

The opioid receptor antagonists μ: Beta-Funaltrexamine (B-FNA), κ: Nor-Binaltorphimine (Nor-BNI), δ: Naltrindole (NALT) and the non-specific opioid receptor antagonist: Naloxone (NAL), and the antiprogesterone mifepristone (Mp) (RU-486:17β-hydroxy-11β-[4-dimethyl-amino-phenyl]-17α-propinyl-estra-4,9-dien-3-one); were obtained from Sigma Chemical Co, St Louis, MO, USA. Tamoxifen citrate (T) was provided by Gador S.A., Buenos Aires, Argentina. L-dihydroxyphenylalanine (L-DOPA) was obtained from Roche, Buenos Aires, Argentina.

Exposure to stress

Rats were placed individually in a jar saturated with ether vapor for 2 min [24,35]. Blood samples were obtained by decapitation 3 min after ether exposure. The experiments were conducted at 9.00 a.m. except on day 3 of pregnancy when they were also conducted at 6.00 p.m. (surge prolactin time). Control rats were always included. Several studies suggest that maximum prolactin response is reached between 2 and 5 min after ether exposure [24,25,35,36]. The order of decapitation did not affect circulating hormone levels of the basal or stressed rats groups.

Blood samples were collected without anticoagulant and allowed to clot at room temperature. Serum was separated by centrifugation and stored frozen at −20 °C until subsequent radioimmunoassay.

Experimental procedures

Experiment 1

This experiment was designed to establish the effect of ether stress on prolactin secretion in NAL-treated rats at late pregnancy. We also included, in the present experiment, two other groups of rats on estrous day and day 3 of pregnancy where the stimulatory effect of the opioid system has been clearly established. Animals on day 19 of pregnancy (late pregnancy), day 3 of pregnancy or estrus day received an *ip* injection of NAL (2 mg/kg) or its vehicle (SAL) at 8.30 a.m. and were sacrificed at 9.00 a.m. Five minutes prior to decapitation, the rats were exposed to ether vapors as described above. Another group of rats was sacrificed following the same schedule at 6.00 p.m. on day 3 of pregnancy when serum prolactin levels are elevated.

It is known that in most situations, prolactin release is under an inhibitory dopaminergic tone of hypothalamic origin. Rats on day 19 of pregnancy were treated with saline or the dopamine precursor L-DOPA (25 mg/kg, *ip*) at 8.15 a.m. to prevent any transient decrease of dopaminergic tone, 15 min later with NAL or saline, and following the same stress exposure they were sacrificed at 9.00 a.m. Blood samples were obtained to determine serum prolactin and progesterone concentrations by radioimmunoassay (RIA).

Experiment 2

This experiment was conducted to study the effect of the fluctuations in progesterone and estrogen action occurring during